A study of the nervous system, neuropeptides and electron transport system of *Hymenolepis diminuta* (Rudolphi, 1819)

SYNOPSIS

Submitted by

BIDISHA UKIL

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Supervisor- Dr. Larisha M. Lyndem

To

Department of Zoology

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Visva-Bharati

Santiniketan- 731235

INTRODUCTION:

Nervous system of helminths consists of neuronal cells, their nerve-nerve junctions as well as nerve innervations to muscles (Geary et al. 1992). Platyhelminths (flatworms) are the earliest diverging phylum of bilateral animals. They are the most primitive organisms with anterior cephalization and a centralized nervous system, features as observed in bilateral organisms. With this neural organization, flatworms have more directive and highly developed motor systems than their radially symmetrical ancestors (Day et al. 1999). Central nervous system (CNS) consists of a brain mainly ganglia from which arise one or more pairs of longitudinal nerve cords connected by commissures peripherally. It is again linked to Peripheral Nervous System (PNS) which consists of smaller nerves and plexuses that innervate tegument, muscles, alimentary and reproductive regions (Ribeiro et al. 2005).

The key feature of nematode nervous system is its simpler organisation compared to platyhelminths. Nematodes possess 300 neurons that are discretely arranged into ganglia associated with anterior (brain, circumesophageal) and posterior (perianal) nerve rings and longitudinal nerve cords (Day et al. 1999). Nematode muscles have neuron like processes that run from muscle to the neuron process bundles in which motor neuron axons reside (White et al. 1986).

Nervous systems of flatworms (Platyhelminthes) and roundworms (Nemathelminths) are well-supplied with neuropeptides that control vital aspects of worm biology (Mousley et al. 2010).

Neuropeptides are small protein like molecules consisting of short stretches of amino acids that function as neurotransmitters, hormones and paracrine factors which convey signals for regulating necessary physiological activities of both vertebrates and invertebrates. Neuropeptides are neuronal signalling molecules that influence the activity of the brain in specific ways. Different neuropeptides are involved in a wide range of brain functions, food intake, metabolism, reproduction, movements etc. Neuronal communication via neuropeptides is pursued by binding of these peptides to specific cell surface receptors. Many neuropeptides are co-released with other small-molecule neurotransmitters. But the major difference between classical neurotransmitters and neuropeptides lies on their mode of synthesis. Classical neurotransmitters are synthesized in nerve terminals, within small vesicles containing necessary enzymes. Neuropeptides in contrast, are synthesized in the cell bodies or soma of peptidergic neurons processed within vesicles by highly specific exo- and endopeptidases from high molecular weight protein precursors and the resulting mature

vesicles are transported along axon to the nerve terminal (Shaw et al. 1996). The findings of homologous peptides in all major invertebrate groups (Platt and Reynolds 1988; de Loof and Schoofs 1990), including flatworms (Halton et al.1990; Fairweather and Halton 1991), suggest that they serve an important regulatory role in all metazoan life.

The most abundant neuropeptide in platyhelminths is the pancreatic polypeptide-like neuropeptide F (NPF), whereas the most prevalent neuropeptides in nematodes are FMRFamide-related peptides (FaRPs) which are also present in platyhelminths. Day et al. 1999 reported the presence of one or two distinct FaRPs in platyhelminths and more than 50 unique FaRPs in nematodes and its bioactivity in platyhelminths appears to be restricted to myoexcitation, whereas both excitatory and inhibitory effects have been reported in nematodes.

Maule et al. 1991 reported the novel parasitic regulatory neuropeptide F consists of 39 amino acid residues terminating in a phenylalaninamide. The full primary structure of the analogous neuropeptide has been determined by Curry et al. 1992, in the free-living terrestrial turbellarian, *Artioposthia triangulata*. The primary structure of both neuropeptides revealed a high degree of homology in their C-terminal regions with 9 of the 17 C-terminal residues in identical positions. Immunocytochemical studies were made by Maule et al. 1991 in the tapeworm *Moneizia expansa,* to localise neuropeptide F. An absolute requirement for binding of the antibody is a C-terminally amidated aromatic residue as well as a penultimate Arg-Pro-Arg tripeptide, indicating clearly that the parasite neuropeptide is C-terminally amidated. Maule et al. 1991 also showed that parasite peptide displays significant homology with the vertebrate pancreatic peptide (PPs). The C-terminal tetrapeptide amide of NPF (Arg-Pro-Arg-Phe-NH2) is identical with that in amphibian and reptilian PPs. Clynen et al. 2009, observed Neuropeptide F in *Caenorhabditis elegans* containing FLP-27 sequences which display the highly conserved GXRMRFamide motif.

Muscular activities and movements not only require proper signalling, but also require energy in the form of ATP to perform necessary physiological and biochemical activities. Energy metabolism is a huge aspect for survival of an organism. According to Saz 1981, many metazoans particularly the parasitic forms, have adapted well to their environments. This is particularly obvious in organisms that reside in anoxic surroundings, such as the large lumen-dwelling intestinal parasites. However, anaerobic energy metabolisms are not confined to parasites in microaerophilic environments but also in highly aerobic surroundings e.g. blood

or lungs. In anaerobic tissues, pyruvate produced during glycolysis is reduced to lactic acid instead of oxidizing to acetyl CoA and CO_2.

Electron transport system of cestodes has been comprehensively studied on *M. expansa* (Cheah 1983) in which its cytochrome chain differs from mammalian system as being branched and possessing multiple terminal oxidases. One branch resembles the classical chain with cytochrome a3 as its terminal oxidase and the other pathway has its terminal oxidase which branch at the level of rhodoquinone or vitamin K. It is an o type cytochrome which is commonly found in microorganisms, parasitic protozoa and plants and it could interact directly with either oxygen or fumarate. Smyth (1989) inferred that cestodes have mechanism for performing respiratory metabolism under both aerobic and anaerobic conditions.

Cytochromes are membrane-bound (i.e. inner mitochondrial membrane) heme proteins containing heme groups and are primarily responsible for the generation of ATP via electron transport chain. They are found either as monomeric proteins (e.g., cytochrome c) or as subunits of bigger enzymatic complexes that catalyze redox reactions. According to Takamiya et al. 1993 contents of cytochromes in the helminth mitochondria show a wide variation depending upon the stage of their life-cycle and habitat of each parasite.

Fujino et al. 1995, reported the differences of activity of cytochrome *c* oxidase in mitochondria in tegument and parenchymal cells of trematodes *Echinostoma trivolvis, Zygocotyle lunata, Schistosoma mansoni, Fasciola gigantica* and *Paragonimus ohirai* which probably reflecting the different energy metabolisms of these worms. Marked aerobic metabolism occurred in *S. mansoni* and *P. ohirai* adults that inhabit host mesenteric veins and lungs respectively. Tegument and parenchymal cells of the former possess relatively few, small mitochondria with tubular cristae which are heavily reactive for cytochrome *c* oxidase while in latter, its activity for cytochrome c oxidase in tegumental mitochondria increased gradually from juveniles to adults, reflecting that the respiratory activity increased with growth and the aerobic metabolism is activated when the worms reach the lung.

According to analyses made by Kita et al. 1996 components and organization of the respiratory chain, change dramatically. Identical to that of the mammalian host reducing equivalents from respiratory substrates such as NADH and/or succinate produced by the TCA cycle are transferred to ubiquinone via the dehydrogenase complexes, NADH-ubiquinone reductase (complex I) and succinate-ubiquinone reductase (complex II), respectively, then transferred to cytochrome c via ubiquinol-cytochrome c oxido-reductase (complex III;

cytochrome bc, complex). Cytochrome c oxidase (complex IV; cytochrome aa3 complex) oxidizes reduced cytochrome c using oxygen as a terminal electron acceptor, and produces H_2O.

ACTIVITIES OF DRUGS:

Drugs acting on neuromuscular system have been extensively studied and can act at any number of sites important in neuromuscular systems. These include the ionic mechanisms involved in signal transmission along nerves; synthesis, release and degradation of neurotransmitters and neurohormones (such as neuropeptides); nerves and muscles receptorsthat recognise these small molecules and second messenger systems and ion channels that mediate the response to the arrival of information (Geary et al. 1992). Trematocidal agents like metrifonate is the only trematocidal agent that acts on neuromuscular system (Holmstedt et al. 1978). The latter could also be an important site for activity of praziquantel since it is found to act on tegumental systems of trematodes (Andrews et al. 1983). Praziquantel has several effects on cestodes. Among them is a pronounced stimulation of muscular contractility at pharmacologically relevant concentrations (Pichard at al. 1982; Andrews et al. 1983). In *Hymenolepis diminuta* praziquantel contracts muscles in absence of external Ca^{2+} from internal pools. In schistosomes, the effect of praziquantel is dependent upon exogenous Ca^{2+} (Pax et al. 1978). Levamisole and pyrantel contract axial muscle at therapeutic concentrations in *Ascaris suum* (Aubry et al. 1970) and piperazine induces reversible flaccid paralysis mediated by a Cl-dependent hyperpolarization of the muscle membrane (del Castillo et al. 1964; Martin 1982). Ivermectin is another drug that inhibits locomotion in *A. suum* (Kass et al. 1980).

Most of the commercially available antinematodal drugs exert their effect on the nervous system of the parasite (Kohler 2001). Levamisole, the tetrahydropyrimidines e.g. pyrantel and morantel and some other structurally related compounds act as acetylcholine agonists. Recent studies have shown anthelmintic acts on the surface of somatic muscle cells of nematodes possess nicotinic acetylcholine receptors (Evans and Martin 1996; Martin et al. 1996, 1998). Binding of these compounds to the recognition site of the excitatory receptor produces depolarisation and spastic paralysis of the nematode muscle that can result in parasite expulsion.

Piperazine, acts as a GABA agonist by opening GABA-gated chloride channels present on nematode somatic muscle cells (Martin et al. 1997a). Binding to these receptors induces an

increase in chloride permeability of the muscle cell membrane that eventually results in a relaxation of the body musculature and flaccid worm paralysis. Oxamniquine and the structurally related hycanthone act on *S. mansoni* by binding covalently to nucleic acids resulting in an irreversible inhibition of the synthesis of these macromolecules (Cioli et al. 1995). The primary target of the salicylanilides and nitrophenols and their selective toxicity in liver flukes, cestodes and blood-sucking nematodes is not well understood. Members of these compounds have been shown to disrupt ATP generation by uncoupling mitochondrial oxidative phosphorylation (Van den Bossche 1985). The potent fasciolicidal sulfonamide derivative, clorsulon, inhibits the activity of two glycolytic enzymes, phosphoglycerate kinase and phosphoglyceromutase, preventing carbohydrate utilisation and its associated substrate-level ATP production in the liver fluke (Schulman et al. 1982).

Helminths have exploited a variety of energy transducing systems in their adaptation to their peculiar habitats in their hosts, such as differences in energy metabolisms are attractive therapeutic targets of helminthiasis eg. NADH-fumerate reductase which is part of unique respiratory system in parasitic helminths (Boveris et al. 1986; Takamiya et al. 1994; Van Hellemond and Tielens 1994; Fioravanti et al. 1998) is inhibited by nafuredin (compound which is also obtained from culture broth of fungus *Aspergillus niger* (FT-0554).

Many phytochemiclas have shown interference with energy generation indirectly in helminths. Synthetic phenolic anthelmintics, like niclosamide, (Martin, 1997b), genistein, an active ingredient of *Flemingia vestita,* and other isoflavone components of the plant have been shown to possess activity against a range of native nematode, cestode and trematode parasites (Yadav et al. 1992; Tandon et al. 1997). Isoflavonoid extracts of the West African Turburku plant, *Millettia thonningii* have activity against different life-cycle stages of *S. mansoni* (Lyddiard et al. 2002). Chrysophanol is a chief constituent of *Cassia tora* showing anthelmintic activities against *Ascaridia galli* (Jain and Patil 2010).

Medicinal value of plants is attributed to only few active constituents that produce a definite physiological effect. Alkaloids, flavonoids, tannins and phenolic compounds form a major group of such bioactive constituent (Edeoga et al. 2005). Plants and herbs have attained a significant role not only as therapeutic agent but also as health maintaining agent (Doshi et al. 2011). Cassia plants are one of such plants that have been used repeatedly in medical science and highlighted in literature for their immense utilities. These plants are distributed mainly in the tropics and subtropics. Extracts of Cassia plants have shown anthelmintic activities against *Raillietina tetragona* and *A. suum* (Kundu and Lyndem 2012; Peter and Deogracious 2006); anti-inflammatory effects against albumin induced paw edema (Doshi et al. 2011) and

also are used to treat certain gastrointestinal disorders (Muffat and Sabbot 1986; Mukhopadhyay et al. 1998).

OBJECTIVES OF STUDY:

- To localise the nervous system and neurosecretory cells of the cestode parasite.
- Identifying significant neuropeptides in the parasite.
- To study the enzymes of electron transport system.
- To observe the effects of anthelmintic drugs as well as natural compounds on the above targets of the parasite.
- In vitro treatment of the parasite for localization of the enzymes

METHODOLOGY:

In order to carry out the above objectives, following methods shall be followed:

Localisation of nervous system of the cestode parasite:

For nervous system, non-specific and specific esterase activity will be studied histochemically following Kemmerling et al. 2006

Localisation of Neurosecretory cells:

Neurosecretory cells in the cestode parasite will be stained histologically and the morphological changes in them upon treatment will be observed.

Identification of Neuropeptides:

Isolation of neuropeptide will be done following the method of Maule et al. 1991. In brief as follows:

a. **Immunocytochemistry:** Immunocytochemical localization of neuropeptides using specific antisera.
b. **Extraction:** Extraction will be done by acid ethanol followed by lyophilisation.
c. **Purification:** Neuropeptide will be purified by sequential chromatographic fractionation, using gel permeation chromatography and reverse phase HPLC.

Isolation of mitochondria:

Mitochondria from the parasite will be isolated following the method by Fioravanti et al. 1992.

Localisation of enzymes:

Enzymes of electron transport system in the parasite will be localised biochemically.

For treatment, crude extract and plant compound will be tested on the parasite:

a. Preparation of plant extracts
b. Treatment of the parasites with plant extracts on the above parameters.

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